Genetic Variation Among Wild and Cultivated Beets of the Section *Beta* as Revealed by RFLP Analysis

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ABSTRACT

The level of genetic variation detected among 7 sugar beet and 4 fodder beet breeding lines was compared to the variation found among 21 accessions of wild beets of the section Beta. RFLP analysis used a set of 32 sugar beet DNA sequences as probes to score a total of 351 bands over all accessions. The band data was used to calculate genetic distances between all pairs of accessions. The distance estimates were subsequently used in a cluster analysis to produce a dendrogram of genetic distances. The analysis unambiguously defined all accessions and clearly defined a fodder beet cluster within the sugar beet cluster. The cultivated beets were all separated from the wild beets. The sugar beet breeding lines showed a considerable amount of genetic variation, comparable with the level of variation detected among the wild beet accessions.

Additional Key Words: Cluster analysis, genetic distance, molecular markers, sugar beet

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Genetic variation is necessary for successful breeding. In a breeding programme it is therefore of utmost interest to quantify the variation among breeding lines and wild relatives of the crop. Until recently, the predominant method for studying genetic variation has been isozyme analysis. However, lately restriction fragment length polymorphism (RFLP) analysis has become the method of choice, since close taxonomic relationships are better resolved. This depends on the ability of RFLP analysis to reveal variation more efficiently than isozyme analysis (Bernatzky and Tanksley, 1989). Furthermore, RFLP markers enable the study of both coding and non-coding regions of the genome (Clegg, 1990), and have a number of other distinct advantages compared to the use of isozymes (Brown, 1992). The greater number of alleles available with RFLP analysis will facilitate the identification of different genotypes and populations. Consequently, RFLP markers have been widely used to estimate and characterize genetic variation in many crop species and a number of RFLP maps have been constructed (Graner and Wenzel, 1992).

Sugar beet has been cultivated for a relatively short period of time and is believed to have an origin, in part, from fodder beet types (Bosemark, 1979; Fischer, 1989). Although hybridization with both *Beta maritima* and various leaf beet types has occurred, the genetic base of sugar beet is assumed to be more narrow than that of most other cross-fertilizing crop species (Bosemark, 1979). Sugar beet varieties are F_1 hybrids that rely on single sources of both cytoplasmic male sterility and the monogerm character. However, Bosemark (1979) argued that sugar beet still retains much variation in its nuclear genes. A study using isozymes (Nagamine et al., 1989) showed the existence of high levels of isozyme diversity among varieties of monogerm and multigerm sugar beet. In addition, the average polymorphism for the 13 isozyme systems studied was lower in fodder beets than in both sugar beet gene pools. In another recent study, Jung et al. (1993) used four sugar beet minisatellite DNA probes to fingerprint cultivated and wild accessions of the genus *Beta*. They concluded that the sugar beet cultivars displayed a low level of genetic diversity, and their analysis did not separate all sugar beet accessions from all *Beta maritima* accessions.

In the present investigation, a set of 32 sugar beet genomic DNA sequences have been used in RFLP analysis to compare the genetic variation present in sugar beet and fodder beet breeding lines with that found in wild beets of the section *Beta*.

MATERIALS AND METHODS

Plant material. To represent the genetic variation present in typical breeding programmes, 3 monogerm (accessions no. 1, 2 and 5) and 4 multigerm (accessions 3, 4, 6 and 7) sugar beet breeding lines were selected from a Swedish breeding programme together with 4 fodder beets from a breeding programme in The Netherlands. The variation among these breeding lines was compared to the variation found among 21 accessions of wild beets of the section *Beta*. The wild beet accessions were sampled along the Atlantic and Mediterranean coasts, covering a large part of the geographic distribution of the section *Beta* (Table 1). The plant material used for RFLP analysis was harvested from one greenhouse-grown plant per accession.

RFLP analysis. Isolation of total genomic DNA from leaf material was carried out according to Saghai-Maroof et al. (1984), with modifications as described by Hjerdin et al. (1993). DNA concentration was determined by fluorometry using Hoechst 33258 as binding dye. Restriction endonuclease digestion, gel electrophoresis, and Southern blotting were carried out according to Hjerdin et al. (1993). The DNA sequences used as probes originate from a sugar beet clone library generated from size fractionated *Pst* I fragments as described by Hjerdin et al. (1993). Clone inserts were amplified from bacterial colonies using polymerase chain reaction with appropriate primers (Sambrook et al., 1989). The amplified fragments were isolated by excision from 1% low-melting agarose gels and labelled by random nona-nucleotide labelling using α-^{32}P-dCTP (3000 Ci mmol^{-1}). Hybridization with labelled probes was done at 60°C in 0.6 x SSC, 10% (w/v) polyethylene glycol, 7% (w/v) SDS, 0.01M sodium phosphate, 0.005M EDTA, and 100 μg ml^{-1} denatured and sonicated salmon sperm DNA (Hjerdin et al., 1993). Membranes were washed in 0.25 x SSC, 0.2% SDS at 60 °C for 45 min, and used to expose autoradiographic film at -70°C.

Data analysis. The RFLP data were analysed as follows: each hybridizing band was treated as a unit character and all accessions were scored for the presence or absence of each band. Similarities in band patterns were then calculated for all pairwise combinations of accessions using the similarity index described by Nei and Li (1979). These similarities were then transformed to distances by subtracting the similarity index from unity. The distance estimates between accessions were then compiled in a standard cluster analysis (UPGMA) using the SAS programme package, to reveal the relationships among the 32 investigated accessions.

Table 1. Source and origin of the accessions used in the RFLP analysis.

No.	Species	Type and origin	Source	Acc. No.
	Sugar beet			
1	*B. vulgaris*	breeding line, Sweden	HABS†	82068875
2	*B. vulgaris*	breeding line, Sweden	HABS	84088627
3	*B. vulgaris*	breeding line, Sweden	HABS	86011975
4	*B. vulgaris*	breeding line, Sweden	HABS	86019231
5	*B. vulgaris*	breeding line, Sweden	HABS	88020987
6	*B. vulgaris*	breeding line, Sweden	HABS	88073057
7	*B. vulgaris*	breeding line, Sweden	HABS	88073060
	Fodder beet			
8	*B. vulgaris*	breeding line, The Netherlands	HBVN‡	89038086
9	*B. vulgaris*	breeding line, The Netherlands	HBVN	89038175
10	*B. vulgaris*	breeding line, The Netherlands	HBVN	89038235
11	*B. vulgaris*	breeding line, The Netherlands	HBVN	90038671
	Wild beet (section *Beta*)			
12	*B. maritima*	collection, Ireland	CGRN§	Z16045
13	*B. maritima*	collection, Great Britain	CGRN	Z16043
14	*B. maritima*	collection, France	CGRN	Z16040
15	*B. maritima*	collection, France	CGRN	Z16041
16	*B. maritima*	collection, Portugal	CGRN	Z16060
17	*B. maritima*	collection, Portugal	CGRN	Z16050
18	*B. maritima*	collection, Portugal	CGRN	Z16055
19	*B. maritima*	collection, Spain	CGRN	Z16065
20	*B. macrocarpa*	collection, Spain	CGRN	Z16068
21	*B. macrocarpa*	collection, Portugal	CGRN	Z16056
22	*B. maritima*	collection, Spain	CGRN	Z16038
23	*B. maritima*	collection, Spain	CGRN	Z16037
24	*B. maritima*	collection, Tunisia	CGRN	Z16088
25	*B. maritima*	collection, Italy	CGRN	Z16083
26	*B. maritima*	collection, Greece	CGRN	Z16077
27	*B. maritima*	collection, Greece	CGRN	Z16076
28	*B. macrocarpa*	collection, Turkey	CGRN	Z16092
29	*B. macrocarpa*	collection	CGRN	Z16094
30	*B. adanensis*	collection, Turkey	CGRN	Z16089
31	*B. atriplicifolia*	collection	CGRN	Z16105
32	*B. orientalis*	collection, India	CGRN	Z16078

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RESULTS AND DISCUSSION

Genomic sugar beet DNA sequences were used as probes in RFLP analysis to discriminate among 32 accessions of wild and cultivated beets of the section *Beta* (Table 1). In a preliminary screening, a large set of sugar beet DNA sequences were hybridized with DNA from 8 sugar beet breeding lines. Based on polymorphism data from this screening, a set of 32 DNA sequences were selected for this study (21 single copy, 8 low copy repeated, and 3 high copy repeated DNA sequences). In total 351 bands were scored among the 32 accessions. The 21 single copy DNA sequences revealed 201 bands of which all were polymorphic across the investigated accessions. A typical example of a single copy sequence hybridized to the 32 accessions is shown in Figure 1. For the low copy repeated sequences, 144 bands were scored with only one non-polymorphic band. For the high copy repeated sequences, only 6 major bands were scored, all of which were non-polymorphic. The individual accessions generally displayed between 50 and 70 bands, with few accessions showing band numbers outside this interval.

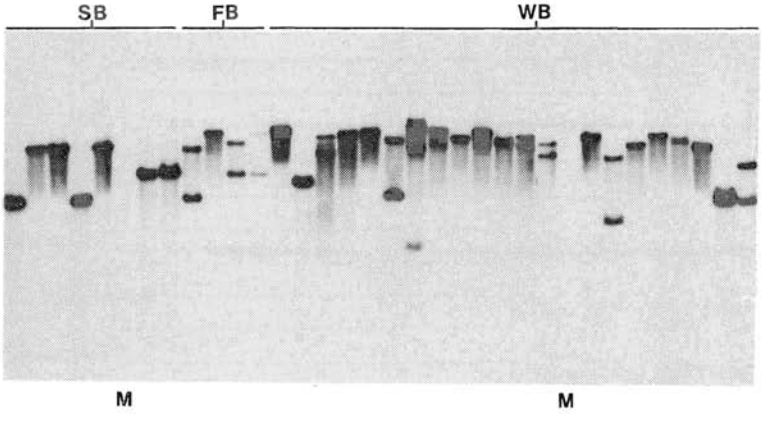

Figure 1. Band differences detected with the sugar beet clone 20P-C08 among the 32 investigated accessions of sugar beets (SB), fodder beets (FB), and wild beets of the section *Beta* (WB).

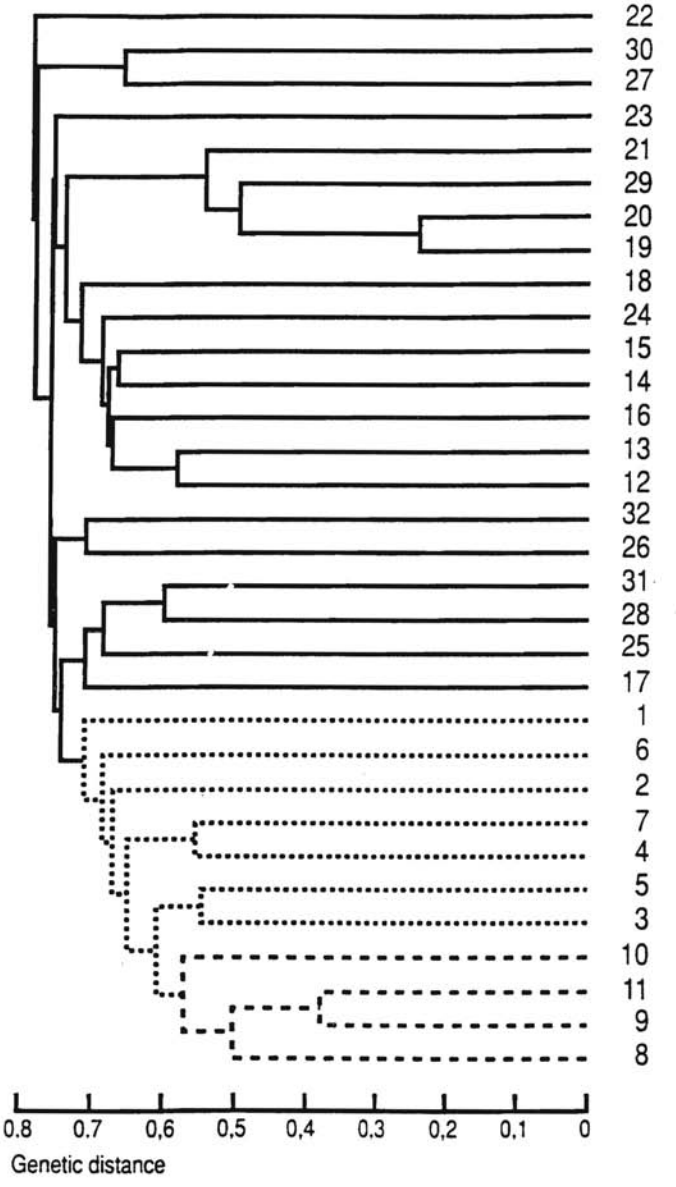

Figure 2. Dendrogram presenting the relationships between the 32 accessions of the section Beta. The analysis is based on cluster analysis of genetic similarity estimates calculated from RFLP data. The accession numbers correspond to those listed in Table 1.

Genetic distances were calculated from the digitized band patterns according to Nei and Li (1979). The result of a cluster analysis based on the genetic distance estimates is presented in Figure 2. The cluster analysis clearly discriminates between the wild and the cultivated beets. Within the cluster containing cultivated beets, the fodder beets form a separate subcluster, showing a lower level of variation than that which generally is detected among the sugar beet breeding lines. The monogerm sugar beet breeding lines do not group together, indicating that, although monogermity has a single genetic origin, the influence of this constraint on the genetic variation is not large enough to be detected in this analysis. Among the wild beets, three of the four *B. macrocarpa* accessions analyzed in this study (nos. 20, 21 and 29), formed a cluster together with a *B. maritima* accession (no. 19). Two of the four accessions in this cluster, the *B. maritima* accession and one of the *B. macrocarpa* accessions (no. 20), originate from the Canary Islands, and show a high genetic similarity. The other two accessions in this cluster, one with a known origin in Portugal, both belong to *B. macrocarpa.* Another cluster is formed by accessions 12-16, 18, and 24. All of these are *B. maritima,* and all except one of the accessions have an origin along the Atlantic coast. Thus, it has been possible to find both taxonomic and geographical associations between the different accessions. In particular, the cultivated beets all cluster together, with the fodder beet accessions showing much less variation than the sugar beet accessions.

Another noteworthy result is the high level of variation observed among the accessions. This variation is expressed as large genetic distances in Figure 2. For the wild beet accessions the genetic distances in most cases vary between 0.8 and 0.5, whereas for the sugar beet accessions the genetic distances vary between 0.7 and 0.5. The fodder beets show smaller distances, in the range of 0.6-0.4. This shows that the breeding lines still retain a considerable amount of the variation that existed in their wild progenitors. There are at least two investigations that support this observation. Nagamine et al. (1989) found high levels of isozyme variation among cultivated beets. However, their measurements of variation, "heterozygosity" and "variation in isozyme phenotypes," cannot be directly compared with our measures of variation. In a study using both cDNA and genomic DNA sequences, Mita et al. (1991) found variation among cultivars to be at the same level as in wild beets. They observed genetic distances somewhat smaller than those in our study. Still, a direct comparison is impossible because they pooled several plants for each accession. Jung et al. (1993) found low levels of variation among sugar beet varieties and substantial genetic variation among wild beets of the section *Beta.* This is in contrast to the

high levels of variation among breeding lines observed here, and reported by Nagamine et al. (1989) and Mita et al. (1991). Possible explanations for this discrepancy are the choice of minisatellite DNA probes and the fact that four plants were pooled for each accession. In contrast, 21 of the 32 probes used in our study were single copy DNA sequences, and instead of pools of plants, we used single plants as representatives for each accession.

In conclusion, the results show that there exists considerable variation among sugar beet breeding lines. This is in agreement with the results presented by Nagamine et al. (1989) and Mita et al. (1991). Thus, there is no reason for immediate alarm concerning an erosion of the genetic variation among sugar beets, and it will probably not be fruitful to make introgressions of exotic germplasm to broaden the genetic base in general. Still, it is clear that certain specific traits, desirable in modern sugar beets, will only be found in exotic germplasm (Van Geyt et al., 1990). Introgression of such traits have been made, e.g. nematode resistance (Jung and Wricke, 1987), and will certainly also be made in the future. Screening for interesting characters and characterization of their inheritance in wild material is therefore of great interest.

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